Pyridin-3-ol in Cigar Smoke

In a continuation of studies on the chemical composition of tobacco leaves[1] and cigar smoke,[2] the weakly acidic substances of such smoke have been investigated. Comparison of the findings with those reported for cigarette smoke[3,4] have revealed that gross qualitative and quantitative differences are evident, including the presence in cigar smoke of a pyridine derivative, pyridin-3-ol, not previously reported in tobacco[4] or its smoke.

Acidic substances were removed from cigar smoke condensate[2] by extraction with aqueous alkali. The alkaline extract was neutralised with carbon dioxide, and the precipitated, weakly acidic substances removed by continuous extraction with diethyl ether. Gas chromatographic analysis of the ether solution revealed several components, including a relatively large major peak which gave a retention time unlike any of the phenols of tobacco or its smoke. Modification of the extraction procedure to include a removal of bases prior to extraction of acidic substances resulted in the disappearance of the substance in the weakly acidic fraction, indicating the amphoteric nature of the unknown. The amount of unknown was estimated to be 130 micrograms per cigar smoked.

The infrared absorption spectrum of the substance indicated the possible presence of a pyridine nucleus and a highly bonded hydroxyl group. Comparison of the spectrum with authentic samples of the isomeric pyridinols showed the spectral characteristics of the unknown to be identical with pyridin-3-ol and unlike the 2- and 4-isomers. It is known that the pyridin-2- and 4-ols exhibit tautomerism[5,6] and the predominating pyridone forms show infrared spectral characteristics of lactams,[7] including the presence in the solid state of a band for the amido group in the 1600-1700 cm.$^{-1}$ region. Since tautomerism is absent and extensive intermolecular bonding is present in pyridin-3-ol,[5] the absence of absorption in the 1600-1700 cm.$^{-1}$ region and the presence of a very broad bonded hydroxyl band centred at 2430 cm.$^{-1}$ make spectrophotometric differentiation of the isomers quite specific. The ultraviolet absorption spectra of authentic pyridin-3-ol and the unknown substance in methanol were identical, having $\lambda_{max.}$ 218 and 278 mμ in contrast to pyridin-2- and 4-ol which show $\lambda_{max.}$ 300 mμ and 257 mμ, respectively.[6]

Additional evidence of identity was obtained by gas chromatographic retention times on two stationary phases (6 mm. by 1·5 m. columns, 20% Carbowax[8] 20 M or 20% neopentyl glycol adipate polyester (NPGA) on Chromosorb W, 200°C., 90 ml. helium per min.) and R_F values from paper chromatographic separations using three solvent systems (A: tert-amyl alcohol-acetate buffer,[9] B: butan-1-ol-benzene-acetate buffer,[10] C: butan-1-ol-pyridine-water[10]). The retention times of authentic and isolated pyridin-3-ol were identical (6·1 mins.) on the one stationary phase (NPGA) which permitted separation of the 2- and 3-isomers. Authentic pyridin-4-ol had a longer retention time than the other isomers on both stationary phases (*e.g.*, NPGA, 17.3 minutes.). On paper chromatograms, R_F values of the unknown were identical with authentic pyridin-3-ol (*e.g.*, Solvent C, 0·91) and different from the authentic 2- and 4-isomers (*e.g.*, Solvent C, 0·72 and 0·52, respectively). The pattern of fluorescence or colour reactions on chromatograms using cyanogen bromide and/or *p*-aminobenzoic acid also confirmed the identity of the unknown.

At least two possible sources of pyridin-3-ol in cigar smoke are evident: dry distillation from the leaf or pyrolysis of a base in the leaf. Postulations of nicotine degradation during fermentation of cigar tobacco contain no pyridinol intermediates in the schemes.[11] However, *in vitro* bacterial degradation of nicotine is believed to involve oxidation of the pyridine nucleus giving 6-hydroxynicotine as the initial intermediate.[12] Extensive studies[13] on the pyrolysis of nicotine have not revealed pyridinols among the pyrolytic products, although many 3-substituted pyridines have been isolated from the mixtures.

This work was supported in part by funds provided by the Cigar Manufacturers Association of America.

Received May 7, 1962

References

1 (*a*) Swain, A. P., Rusaniwskyj, W. & Stedman, R. L., *Chem. & Ind.*, 1961, 435; (*b*) Stedman, R. L., Swain, A. P. & Rusaniwskyj, W., *Tobacco Science*, 1962, **6**, 1

2 Schmeltz, I. & Schlotzhauer, W. S., *Tobacco Science*, 1961, **5**, 92

3 (*a*) Bentley, H. R. & Berry, E. G. N., "The Constituents of Tobacco Smoke: An Annotated Bibliography," 1959, *p.* 28 (London: Tobacco Manufacturers Standing Committee); (*b*) Izawa, M., Kobashi, Y. & Sakaguchi, S., *Bull. agr. chem. Soc. Japan*, 1959, **23**, 194; (*c*) Carruthers, W. & Johnstone, R. A. W., *Nature, Lond.*, 1960, **185**, 762; (*d*) Hoffmann, D. & Wynder, E. L., *Beitr. z. Tabforsch.*, 1961, **3**, 101

4 Johnstone, R. A. W. & Plimmer, J. R., *Chem. Rev.*, 1959, **59**, 885

5 Gibson, J. A., Kynaston, W. & Lindsay, A. S., *J. chem. Soc.*, 1955, 4340

6 Mosher, H. S., "Heterocyclic Compounds," (Elderfield, R. C., ed.), Vol. 1, 1950, *pp.* 435–443 (New York: John Wiley and Sons Inc.)

7 Bellamy, L. J., "The Infrared Spectra of Complex Molecules," 2nd ed., 1958, *pp.* 203, 214, 218, 281 (New York: John Wiley and Sons Inc.)

8 Mention of commercial products does not imply endorsement by the U.S. Department of Agriculture over similar products not named

9 Tso, T. C. & Jeffrey, R. N., *Arch. Biochem. Biophys.*, 1953, **43**, 269

10 Kuffner, F., Schick, K. & Bühn, H., *Monatsh.*, 1956, **87**, 749

11 Frankenburg, W. G., Gottscho, A. M. & Vaitekunas, A. A., *Tobacco Science*, 1958, **2**, 9

12 Hochstein, L. I. & Rittenberg, S. C., *J. biol. Chem.*, 1959, **234**, 156

13 (*a*) Woodward, C. F., Eisner, A. & Haines, P. G., *J. Amer. chem. Soc.*, 1949, **66**, 911; (*b*) Jarboe, C. H. & Rosene, C. J., *J. chem. Soc.*, 1961, 2455